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Invited Review

Anthocyanin composition and content of blueberries from around the world

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Abstract. *Background*: The phytochemical content of blueberries, particularly anthocyanins and other polyphenols is of increasing importance to researchers in the field of food and health, because they are thought to be largely responsible for the health benefits of this popular fruit.

Objective: To determine the potential for selective breeding of blueberries to produce high-polyphenol and particularly, high anthocyanin cultivars, while retaining desirable traits such as high yield, disease-resistance and large fruit.

Methods: Comparison of content data for the anthocyanin and polyphenol classes of phytochemicals, in blueberries, between a comprehensive collection of literature reports and data from the New Zealand blueberry breeding programme.

Results: There was a wide range of variation in anthocyanin and total polyphenol content both between cultivars in a given growing region and within the same cultivar, when grown in different regions. Experience from the New Zealand breeding programme suggests that selection based on critical agronomic traits, such as yield, or fruit size, but not including anthocyanin content, tends towards a marked reduction in this trait.

Conclusions: There is potential to selectively breed cultivars with high anthocyanin content, but it appears that this trait must be included in the selection parameters, if it is to be maintained or enhanced.

Keywords: Anthocyanin, blueberry, polyphenol content, climate, breeding

1. Introduction

Blueberries are regarded as "superfruits", which are thought to provide many health benefits beyond nutrition [1, 2]. Polyphenolic compounds are thought to be the major health-promoting compounds in plant foods, because they are potent *in vitro* antioxidants and more recently, many other potential health benefits have been found that are unrelated to antioxidant capacity. These benefits include reduced incidence of the major modern diseases cardiovascular disease, diabetes and cancer [2–4], as well as mechanistic properties that contribute to the epidemiological benefits, such as management of inflammation [2, 4], stimulation of antioxidant and xenobiotic metabolising enzymes (thus reducing DNA and protein damage by free radicals and toxicity of xenobiotics) [3] and augmentation of the effects of exercise [5, 6]. Anthocyanins are the major polyphenols in blueberries and this group of phytochemicals is thought to be

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Table 1

Mean content and range (min-max) of individual anthocyanins over 80 blueberry genotypes from the Plant & Food Research (PFR) *Vaccinium* repository – Ruakura Research Centre – New Zealand (37°48′S 175°17′E). Data reported are from evaluation of fruit between the seasons 2009 and 2012 and are sorted by mean content

Compound	Mean content (mg/100 g)	Range (mg/100 g) (Min-max)
Total anthocyanin content	199	(57–503)
Total malvidin	79.4	(16–198)
Mv[1]-Gal/Pn-Glu[2]	39.1	(5.5–84.1)
Chlorogenic acid	36.9	(1.3–171)
Mv-Glu/Pn-Ara	28.1	(0.1–61.7)
Dp-Glu/Cy-Gal	27.5	(0.1–118)
Dp-Gal	25.6	(0.9–62.8)
Quercetin-glycosides	22.7	(4–57.2)
Pet-Gal/Cy-Ara	21.9	(7.7–59.3)
Mv-Ara	19.8	(6.4–52)
Pet-Glu	16.6	(1.4–38.2)
Cy-Glu/Dp-Ara	15.8	(5.2–39.1)
Pet-Ara/Pn-Gal	14.8	(4.8–30.1)
Acetylated anthocyanin	11.6	(0.1–34)

[1] Cy = cyanidin, Dp = delphinidin, Pt = petunidin, Mv = malvidin, Ara = 3-arabinoside, Gal = 3-galactoside, Glu = 3-glucoside. [2] Where 2 species are listed together, this indicates that they cannot be separately quantified by a high-through put (i.e., limited peak resolution) HPLC method, usually because a minor species is hidden by a major one, e.g., Pn glycosides are hidden by much larger peaks of the usual major species Mv.

responsible for many of the health benefits of berry consumption [7–10]. This considerable interest in anthocyanins and health has resulted in a drive to breed, or find naturally, new berry cultivars with ever higher anthocyanin content, in addition to the usual requirements of commercial cultivars such as yield, taste, disease resistance. In this article, we review the literature on the anthocyanin content of blueberries and compare these data with previously unpublished data from New Zealand. A recent goal of the blueberry breeding programme at Plant & Food Research (PFR) in New Zealand is to increase the content of health-promoting phytochemicals, especially anthocyanins, while maintaining the fruit quality and agronomic traits.

2. Anthocyanin composition of blueberries

There have been many in-depth studies of this subject, as well as some excellent reviews, so we will briefly summarise what others have already covered very well. Blueberries (Fig. 1) contain a wider range of anthocyanidins than most types of berries, with malvidin predominating and similar concentrations of delphinidin, cyanidin, petunidin and pelargonidin [11–15]. The glycosidic forms found in blueberries are glucosides, galactosides and arabinosides, with all possible permutations being found in at least some cultivars, although relative proportions vary widely and in some cultivars may be absent altogether. Blueberries contain a significant proportion of acylated anthocyanins, primarily the *p*-coumaroyl and acetyl esters on C-6 of the various sugar residues [16, 17]. The proportion of acylated species is highly variable among different cultivars, with some having barely detectable amounts [11, 12, 14, 18].

According to a recent survey on anthocyanin composition of fruit from the blueberry repository at PFR (different *Vaccinium* species), malvidins were the largest group of anthocyanins (Table 1). The results reported are from multiple year evaluation of fruit composition of over 80 genotypes and as expected, significant seasonality variation and significant differences among genotypes were found (Scalzo, unpublished results). The results reported in Table 1 are expressed as mean content for each of the specific compounds and the range of variation is also reported.

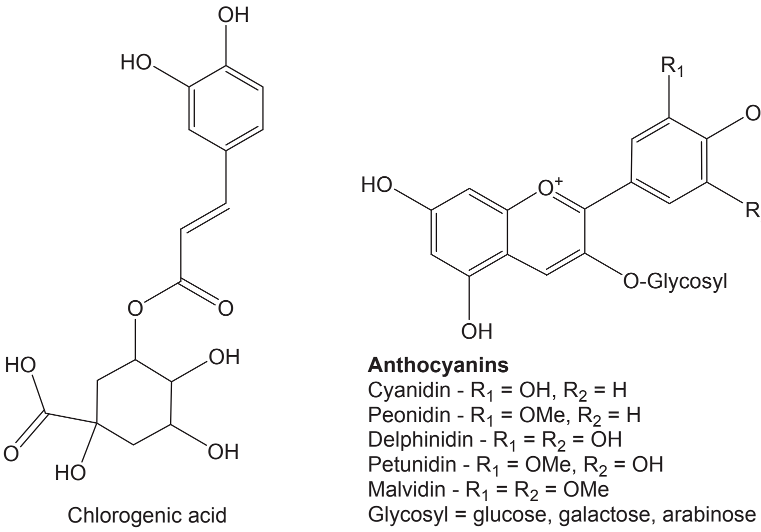

Fig. 1. Structures of the main polyphenols found in blueberries.

3. Other polyphenols

Blueberries contain varying amounts of other polyphenols that make up the "Total Polyphenols" measurement [19]. The only major compound, however, is chlorogenic acid (Fig. 1), which may be present at a high concentration [19]. It is accompanied by small amounts of quercetin glycosides [17, 19].

4. Variation in anthocyanin and phenolic content between cultivars and growing regions

Blueberry fruit available from the market (*Vaccinium corymbosum, V. corymbosum* hybrids*, V. virgatum, V. angustifolium*) come from cultivars that have been selected for specific agronomic and fruit traits (i.e. high yield, disease resistance, large fruit size, and abundance of wax coating on the fruit that gives a light blue "bloom"), and not necessarily for the concentration of health-promoting phytochemicals in the fruit. Within the *Vaccinium* species, there is a large range of cultivars available to growers. Some are new releases from recent breeding programmes and are readily available from nurseries. Others are more traditional cultivars that have been grown for many years and are well adapted to particular environments. Two or more different cultivars grown in the same commercial block is a popular choice among growers, to satisfy pollination requirements and to extend the production window from early to late season fruit. There is considerable variation in anthocyanin and total polyphenol content between cultivars (Table 2), which suggests that there is considerable scope for breeding programmes to generate new cultivars with above-average anthocyanin and polyphenol contents.

5. Comparison with New Zealand content data

A survey of the variation of anthocyanin content in blueberry fruit of different cultivars from the Plant & Food Research (PFR) *Vaccinium* germplasm repository was carried out to find potential sources of enhanced concentrations of anthocyanins. Fruit of recently released PFR cultivars were also screened and compared with the germplasm, and a summary of the results is shown in Table 3. We found that the lowest range of anthocyanin content was found in *V. corymbosum hybrids* of the southern highbush type, while the highest range of anthocyanin content was found in fruit of *V. virgatum*. Within blueberry species we found great variation in anthocyanin content between cultivars (Table 3), in agreement with previous literature (Table 2). In our cultivar collection, the difference in total

Table 2

Summary of literature reports on anthocyanin and total polyphenols content of blueberries

Location	Genotype	Anthocyanins mg/100 g fruit	Total phenolics mg/100 g fruit
Northwest Romania [25]	'Elliott'	163	526
	'Bluecrop'	161	652
	'Duke'	101	424
USA, various regions [16]	A-98	369	–
	'Bluecrop'	144	–
	'Ozarkblue'	144	–
	US-497	823	–
	US-720	432	
USA, various regions [17]	A-98		359
	'Bluecrop'	–	227
	'Ozarkblue'	–	254
	US-720	–	370
	US-729	–	324
Arkansas, USA [11]	A-4	128	241
	A-12	145	550
	A-23	304	316
	A-179	338	337
	A-259	134	688
	A-272	135	640
	A-439	59	581
	'Bluecrop'	106	338
	'Cape Fear'	177	304
	'Climax'	174	406
	'Duke'	172	327
	'Georgiagem'	124	283
	'Magnolia'	73	471
	'Ozarkblue'	156	379
	'Reveille'	137	307
	'Summit'	85	287
	'Tifblue'	170	451
	US 497	178	867
	US 729	372	632
Northwest Croatia [26]	'Duke'	165	358
	'Elliott'	152	476
	'Sierra'	240	528
	'Bluecrop'	120	368
Arkansas, USA [20]	'Bluecrop'	182	–
	'Brigitta'	165	–
	'Cape Fear'	255	–
	'Duke'	216	–
	'Georgiagem'	156	–
	'Magnolia'	116	–
	'Nui'	122	–
	'Ozarkblue'	158	–
	'Reka'	149	–
	'Reveille'	144	–
	'Rubel'	325	–

Table 2

Continued

Location	Genotype	Anthocyanins mg/100 g fruit	Total phenolics mg/100 g fruit
Nova Scotia, Canada [34]	'Blomidon'	121	–
	'Chignecto'	261	–
	'Cumberland'	135	–
	'Fundy'	211	–
	Wild berries	147	–
	'Bluecrop' 1	112	–
	'Bluecrop' 2	109	–
Italy [35]	'Goldtraube'	104	251
	'Patriot'	92	310
	'Bluecrop'	129	299
	'Darrow'	126	298
	Wild berries lot 1	330	577
	Wild berries lot 2	344	614
Arkansas, USA [23]	US 497	245	580
	US 720	240	390
	'Cape Fear'	170	400
	'Ozarkblue'	90	310
	'Magnolia'	75	270
	'Bluecrop'	72	305
	'Summit'	49	298
Nova Scotia, Canada [12]	'Blomidon'	95	–
	'Cumberland'	153	–
	'Fundy'	255	–
	'Bluecrop'	83	–
	'Coville'	100	–
	'Jersey'	117	–
	Wild berries lot 1	202	–
	Wild berries lot 2	250	–
	Wild berries lot 3	166	–
Warsaw, Poland [24]	'Bluecrop'	175–184	–
Greater Hinggan Mountains, China[36]	Wild blueberries	603	177
Aurora, Oregon [22]	'Bluegem'	242	717
	'Bluecrop'	84	304
	'Brigitta Blue'	103	246
	'Duke'	173	274
	'Rubel'	269	435
	'Summit'	73	211
	'Summit' II	119	369
USA, Canada, various regions [21]	'Bluecrop'	93	190
	'Jersey' (Oregon)	101	181
	'Jersey' (Michigan)	100	206
	'Jersey' (New Jersey)	117	221
	'Croatan'	119	275
	'Duke'	127	306
	'Rancocas'	141	317
	'Rubel'	235	390

Table 2

Continued

Location	Genotype	Anthocyanins mg/100 g fruit	Total phenolics mg/100 g fruit
La Araucanía Region, Chile [37]	'Bluegold'	206	433
	'Brigitta'	468	190
	'Legacy'	227	570
Georgia, USA [27]	'Austin'	178	669
	'Brightblue'	16	929
	'Brightwell'	87	387
	'Climax' (early June)	105	288
	'Climax' (irrigated, late June)	197	641
	'Climax' (non-irrigated, late June)	99	270
	'Premier'	157	522
	'Sharpblue'	129	533
	'Tifblue'	108	392
	'Woodard'	117	623
New Jersey, USA [38]	'Bluecrop', organic	131	319
	'Bluecrop', conventional	82	190
Alabama, USA [28]	'Powderblue' organic	181	
	'Powderblue' conventional	180	
	'Climax' organic	222	
	'Climax' conventional	256	
Hangzhou, China [29]	'O'Neal'	70	204
	'Misty'	101	195
	'Sharpblue'	30	175
	'Powderblue'	243	256
	'Gardenblue'	92	413

anthocyanin content of fruit between the cultivar with the lowest content ('Puru') and the one with the highest content ('Ono') is 7-fold. Both cultivars were released from the PFR blueberry breeding programme and were selected for attributes other than phytochemical composition of fruit (i.e., flavour, fruit size, productivity). Our cultivar collection includes PFR cultivars that were bred in New Zealand and therefore selected to be adaptable to specific environments. Some of those cultivars such as 'Reka' and 'Nui' have been widely grown around the world and the total anthocyanin content of their fruit in North America has been reported [20], as 149 and 122 mg/100 g fresh fruit, respectively. The difference in the anthocyanin content of the same cultivars growing in New Zealand is marked, 'Reka' being much lower, at 92.6, whereas 'Nui' is higher, at 148.9 mg/100 g. There are several other examples where the growing environment may have affected the anthocyanin content of fruit. Standard varieties such as 'Rubel', 'Bluecrop', 'Duke','Elliott', 'Climax' and 'Powderblue' are widely cultivated around the world and often integrated into cultivar and germplasm collections and referred to as "benchmark varieties". The anthocyanin content in 'Rubel' was variable between locations but consistently high, ranging from 235 [21] to 325 mg/100g[20]. In each of the cultivar collections reported by different authors, 'Rubel' was the cultivar with the highest anthocyanin content [22]. The anthocyanin content in 'Bluecrop' varies greatly between regions, ranging from 72 [23] to 184 mg/100 g [24]. Similarly, 'Duke' anthocyanin content ranges from 101 [25] to 216 mg/100 g [20], 'Elliott' from 152 [26] to 261 mg/100 g (Table 3), 'Climax' from 99 [27] to 256 mg/100 g [28] and 'Powderblue' from 165 (Table 3) to 243 mg/100 g [29]. The total phenolic content in 'Rubel' was also variable between locations, but consistently high [21]. The phenolic content in 'Bluecrop', 'Duke', 'Elliott' and 'Climax' varied greatly between regions but it was consistently higher than their total anthocyanin content (Table 2), whereas in 'Powderblue' fruit the two traits were similar [29].

An alternative explanation to regional or seasonal factors in these marked variations might be differences in analytical methods or extraction protocols. In choosing literature data, we selected only those reports which used

Table 3

Anthocyanin and total phenolics content in blueberry fruit of different genotypes from the Plant & Food Research (PFR) *Vaccinium* repository – Ruakura Research Centre – New Zealand (37°48′S 175°17′E). Cultivars are grouped by species and sorted by anthocyanin content

Cultivar	Anthocyanins mg/ 100 g fruit	Total phenolics mg/ 100 g fruit
Northern highbush (V. corymbosum)		
‘Rubel’	290.0	388.9
‘Darrow’	286.8	
‘Elliott’	261.3	363.3
‘Northland’	238.7	287.6
‘Duke’	189.6	302.8
‘Jersey’	184.5	220.0
‘Caroline Blue’	153.6	–
‘Nui’*	148.9	214.5
‘Hortblue Poppins’*	138.9	392.9
‘Blue Moon’*	138.9	240.5
‘Weymouth’	120.2	–
‘Sunset Blue’*	106.8	164.7
‘Brigitta’	101.8	199.2
‘Nelson’	99.9	–
‘Reka’*	92.6	323.2
‘Elizabeth’	89.8	307.3
‘Bluecrop’	83.1	714.3
‘Puru’*	45.9	188.7
Mean Northern highbush	*153.97*	*307.7*
Southern highbush (V. Corymbosum hybrids)		
‘Island Blue’*	249.6	226.9
‘Marimba’	148.6	178.2
‘Blue Bayou’*	139.5	224.3
‘O’Neal’	138.9	201.9
JU83	136.0	188.1
‘Misty’	126.3	–
‘Ozarkblue’	80.9	–
Mean Southern Highbush	*145.69*	*203.9*
Rabbiteye (V. virgatum)		
‘Ono’*	347.5	388.8
‘Centurion’	275.5	422.9
‘Southland’	266.2	372.1
‘Maru’*	263.8	328.7
‘Brightblue’	247.3	–
‘Dolce Blue’*	238.2	392.1
‘Climax’	234.1	–
‘Little Giant’	230.7	–
‘Centra Blue’*	212.0	378.5
‘Velluto Blue’*	192.5	311.4
‘Ocean Blue’*	189.0	301.7

Table 3
Continued

Cultivar	Anthocyanins mg/ 100 g fruit	Total phenolics mg/ 100 g fruit
'Rahi'*	168.5	277.2
'Sky Blue'*	167.2	264.7
'Powderblue'	165.3	346.5
'Tifblue'	145.3	366.0
'Delite'	111.9	333.5
Mean Rabbiteye	*215.94*	*344.9*

*PFR cultivars developed in New Zealand.

similar methods to ours [30], i.e., solvent extraction of fresh fruit, followed by HPLC measurements, calibrated using authentic standards. Some reports involved extraction of dried fruit and are therefore not comparable. In addition, we have found marked seasonal variation in anthocyanin content, even comparing fruit from the same bushes in PFR research orchards and analysing by the same methods in the same laboratory (unpublished results). It appears, therefore, that differences arising from methodology are relatively insignificant compared with environmental differences.

6. Breeding blueberries for high anthocyanin content

Connor et al. [31] reported statistically significant genotype × environment interactions for both total anthocyanin and phenolic content; however, they did not discuss the differences between regions that may be caused by horticultural practices and climatic factors, such as differences in ultraviolet intensity, temperature during fruit ripening, water stress, mineral nutrients available.

When comparing density plots of the total anthocyanin and total phenolic contents of PFR *Vaccinium* genotypes growing in the same environment, we found a marked difference in distribution between germplasm, selections and seedlings (Fig. 2). For total anthocyanin and total phenolic content, neither the seedlings nor selections were well represented at the high end of total anthocyanin or total phenolic content, the highest concentrations being found in germplasm (Fig. 2). The germplasm collection in our study included small berry cultivars such as 'Rubel' and according to our previous studies (Scalzo, unpublished results), a negative correlation was found between fruit size

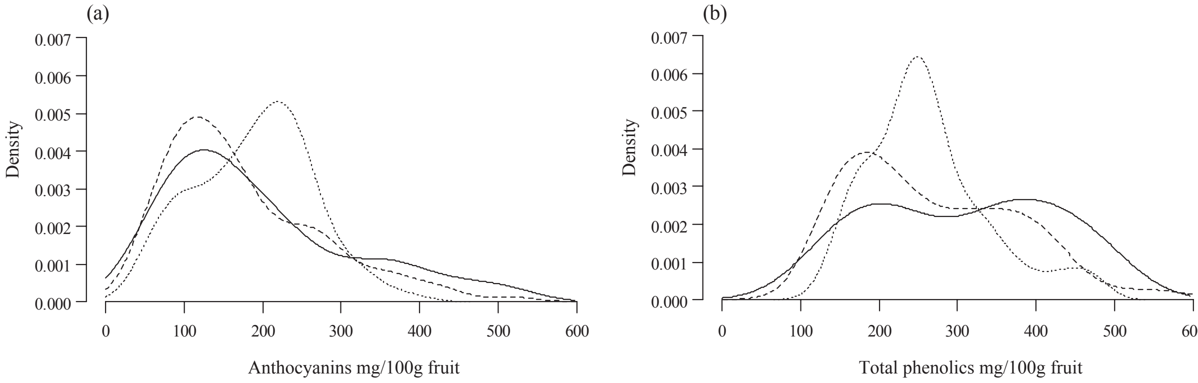

Fig. 2. Density plots for (a) total anthocyanins and (b) total phenolic compounds for blueberry germplasm (——), selections (- - - -) and seedlings (·····) grown in New Zealand between the seasons 2009 and 2012. There were 25 observations for the germplasm, 300 for the selections and 80 for the seedlings.

and total anthocyanin content (–0.48) and between fruit size and total phenolic content (–0.50). Anthocyanins are mostly concentrated in the blueberry skin; therefore, when comparing the same quantities of fruit samples that have different sizes, the small sized fruit will have a relatively higher skin area and consequently, higher anthocyanin content.

The PFR selections surveyed in this study included breeding material that was selected for the hand-harvest fresh fruit market and as such, a great deal of effort was put into selecting material with suitable fruit traits such as large size, high firmness, light blue colour and small pedicel scar [32]. High anthocyanin content was one trait not included in the selection process. Progress towards increasing anthocyanin content was made, however, when a new breeding population was developed, with the aim of introducing high anthocyanin and phenolic content into the fruit (Fig. 2). The estimated mean for the seedling population for both the traits (total anthocyanin and total phenolic contents) was higher than that for the selections.

Several generations may be needed to introgress the high anthocyanin and polyphenolic content in our breeding populations; however, Connor et al. [31] reported a moderate heritability for total anthocyanin content of 0.56, which indicates that breeding progress can be expected and they [33] indicated that selecting parents on the basis of their observed anthocyanin content should be moderately successful.

7. Conclusion

The information collected here shows that there is considerable international interest in studying and enhancing the content of blueberry polyphenols. We found some inconsistent results when comparing anthocyanin and phenolic content of varieties between locations. The existence of variability is fundamental for genetic improvement; however, the true source of variation has to be readily identified, as the environment appears to influence the phenotype greatly. A cultivar with exceptional anthocyanin content in one growing region may perform poorly in another.

Our collection showed great inter-species variation, and we have identified sources for genetic improvement for anthocyanins and polyphenolics from our germplasm and cultivar collections. A well-focused breeding programme can create new cultivars specifically selected for improved fruit phytochemical content. Our initial selection population was created for genetic improvement of fruit traits other than phytochemical content and resulted in a lower phytochemical content in the fruit. However, when we introduced parents with high fruit anthocyanin and polyphenolic contents, we started to make progress towards an increased phytochemical content, compared with that of the previous generation. The breeding population that was created with the intention of improving the anthocyanin and phenolic contents has been successful in this regard, and since the anthocyanin content is a moderately heritable trait, we plan to continue the selection of parents for producing further generations.

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